

Isolating *Plasmodium falciparum* proteins using Saponin Lysis for SDS-PAGE

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Reagents

- At least a 5% parasitized culture
- Sterile PBS
- 10% Saponin/PBS made fresh
- Protein gel loading buffer

Materials

- Conical tubes
- Allegra® 6 Series and GH-3.8
Swinging Bucket Rotor
- Eppendorf tubes

1. Transfer your parasite culture to a conical tube. Harvest cells 1300 rpm, 4 min, 4°C low brake.
2. Aspirate the supernatant, and wash *i*RBCs in the volume of culture you started with using sterile PBS. Pellet the cells by centrifugation at 1,870 RPM (800xg), 5 min 4°C low brake.
3. Aspirate supernatant. Resuspend *i*RBCs with the same volume of sterile PBS. Lyse *i*RBCs by adding an appropriate volume of 10% saponin for a final concentration of 0.1% saponin.
4. Gently invert the tube to mix the cells and incubate for ~2 minutes at room temperature.
5. Spin 2,800 RPM (1800xg), 10 minutes 4°C low brake.
6. Aspirate the supernatant and resuspend the parasites and associated membranes with the same volume of PBS you have been using.
7. Spin 2,800 RPM (1800xg), 10 minutes 4°C low brake. Aspirate the supernatant. Using 200 µl of protein gel loading buffer resuspend pellet and transfer to an Eppendorf tube.
8. Boil sample at 100°C for 5 minutes. Load onto gel, or freeze sample at -20°C until ready to use.